

# Alternative Strategies to Control *Salmonella* in Poultry

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## Take-Home Message

Foodborne salmonellosis is one of the major causes of gastrointestinal illness in the United States. Recent reports indicate that ~ 1 million cases of non-typhoidal salmonellosis occur in the US, resulting in an estimated \$4.4 billion loss annually due to hospitalizations and deaths. Poultry and poultry products are epidemiologically-linked sources for *Salmonella* transmission to humans. *Salmonella* colonizes in the cecum of chickens, resulting in excretion of the pathogen to the environment, leading to carcass contamination during slaughter and contamination of egg shells during oviposition. Controlling *Salmonella* colonization in poultry would reduce contamination of poultry products and potentially decrease foodborne infections in humans. Several on-farm approaches have been investigated to control *Salmonella* with varied success rates. Strategies that are safe to poultry, humans and the environment, and adoptable by poultry producers are required. Our research focuses on the use of natural and environmentally-friendly molecules supplemented through feed to control *Salmonella* colonization in poultry. An overview of our research on such alternative strategies, especially with phytophenolics and fatty acids, are discussed in this paper. Our applied and molecular research has revealed high potential for these natural molecules against the major serotype of *Salmonella*, namely *S. Enteritidis*. Unique properties of these natural antimicrobial agents may qualify them for inclusion in commercial poultry diets.

## Issue of *Salmonella* infections

Despite several control methods, foodborne *Salmonella* remain one of the most common causes of human enteric diseases in the United States (US) and worldwide. The role of animal-derived foods, especially poultry in human salmonellosis has been a matter of serious discussion and research in the recent decades. According to the United States Department of Agriculture (USDA), food-associated *Salmonella* infections cost an estimated \$3 billion annually (USDA, 2009). Recent reports indicate ~1 million cases of non-typhoidal salmonellosis occurring in the country resulting in an estimated \$4.4 billion loss annually due to hospitalizations and deaths (Scallan et al., 2011; Scharff, 2012). Responding to this situation, the US Department of Health and Human Services has set a goal of reducing *Salmonella* incidence by 25% by 2020 (Jackson et al., 2013). Several serotypes of *Salmonella*, including *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg*, *S. Hadar* and *S. Newport* cause enteric disease in humans. However, among the 45,828 laboratory confirmed foodborne *Salmonella* serotyped in 2011, *S. Enteritidis* was the most common serotype followed by *S. Typhimurium* and *S. Newport* (CDC, 2011). The report also noted that among the top serotypes reported to the National Veterinary Services Laboratories for typing, *S. Enteritidis* was isolated from 67.4% and 92.1% of



chicken samples obtained from clinical and non-clinical sources, respectively (CDC, 2011), underscoring the potential role of poultry in *Salmonella* infections.

### ***Salmonella* impacts global trade of US poultry**

The US is one of the leading producers of excellent quality poultry and is regarded as a global leader in poultry exports. Recent estimates indicate an increase in the value of chicken sales and broiler production by 11 and 24%, respectively, compared to the values in 2012 (US Poultry and Egg Association, 2014). However, notable trade issues on the delivery of microbiologically safe poultry products to the consumers have increasingly limited export and sales. This is critically important since food safety issues not only impact consumers domestically, but affect rest of the world poultry markets as well. Many countries and a few US states that import American poultry have developed stricter rules for trade recently. For example, in 2013, Mexico banned import of poultry meat products from three farms in California implicated in *Salmonella* outbreak (Food Safety News, 2013). In light of the recent outbreak of salmonellosis, Russia is reportedly considering import restrictions on American poultry (Vorotnikov, 2014).

### **Live poultry and poultry meat as sources for foodborne *Salmonella* outbreaks**

*Salmonella* spp. can colonize the intestinal tract of poultry. The pathogen colonizes predominantly the cecum, further contaminating the environment, chicken carcass, and eggs at or after lay. There is mounting evidence that contaminated poultry products, especially meat and eggs are epidemiological sources for *Salmonella*, especially *S. Enteritidis* (Humphrey and Jorgensen, 2006; Marcus et al., 2007). The USDA-FSIS data on *S. Enteritidis* in broiler chicken carcass rinses collected from 2000 to 2005 indicated substantial increase in the annual number of isolates and the proportion of establishments with *S. Enteritidis*-positive rinses (Altekruse et al., 2006). Later, White et al. (2007) reported isolation of *Salmonella* from 26.4% of ground turkey, 22.5% of ground chicken, and 11.2% of broiler samples tested in the study (N=12,699/293,938 samples positive for *Salmonella*), with the largest number of *S. Enteritidis* isolates recovered from broiler carcasses. In addition to *S. Enteritidis* outbreaks, other serotypes of *Salmonella* have also been increasingly reported causing foodborne outbreaks. For example, in 2011, a multistate outbreak of *S. Heidelberg* involving consumption of contaminated kosher broiler chicken livers was reported to cause 190 human illnesses. In addition, *S. Altona* and *S. Johannesburg* were identified as causative agents in two independent outbreaks sourced to chicks and ducklings causing illness in around 100 persons. Another serotype, *S. Hadar* was isolated from turkey burgers (CDC, 2014). Recently, other serotypes of *Salmonella*, namely *S. Montevideo*, *S. Infantis*, *S. Newport* and *S. Lille* have been isolated from live poultry (CDC, 2014). These data indicate that several serotypes of *Salmonella* harboring in chickens could potentially result in foodborne infections in humans.

### ***Salmonella* employs multiple strategies for colonization in chickens**

In order to establish a successful ecological niche in chickens, *Salmonella* has to bypass several hurdles in a rather hostile environment. It has to tolerate differences in the growth conditions outside and within the avian host. The pathogen has to adjust to differences in temperature, osmolarity, oxidation-reduction potentials, iron concentrations, acidity, organic and inorganic nutrient environments, antimicrobial substances, host immune response, peristalsis, mucus and several other microenvironments (Slauch et al., 1997). Most of *Salmonella*'s capabilities for establishing colonization such as motility, adhesion to host cells and invasion of epithelial cells are critical for the pathogen persistence within the host. Most of the virulent *Salmonellae*, including *S. Enteritidis* employ mechanisms such as resistance to lytic action of complement (D'Aoust, 1991), up-expression of siderophores (that accumulate iron for growth),



virulence plasmids (Slauch et al., 1997), cytotoxins, diarrheagenic enterotoxins (D'Aoust, 1991), and by antimicrobial resistance (Travers and Barza, 2002). Moreover, *Salmonella* has to operate nutrient utilization strategies that are totally or significantly different from those employed by a much larger number of endogenous bacteria consisting of obligate anaerobes, or adopt strategies to find new ecological niches where they need not compete with the normal flora (Dhawi et al., 2011). It is highly likely that *Salmonella* tends to colonize close to the mucosa, where nutrients and oxygen are present at very high concentrations that will not allow obligate anaerobes to colonize (Poulsen et al., 1995). In addition, they have to utilize available electron receptors (Gennis and Stewart, 1996) and adopt different patterns of nutrient metabolism (Pullinger et al., 2008).

Although multiple serotypes of *Salmonella* associated with poultry are involved in causing human infections, *S. Enteritidis* is the most genetically homogenous serotype (Porwollik et al., 2005; Swaminathan et al., 2001). Although the genomic diversity is limited within the serotype, the field isolates vary in their virulence, survival and colonization capabilities (Clavijo et al., 2006; Jain and Chen, 2007; Yim et al., 2010). Despite variations in the virulence characteristics, on-farm investigations have revealed that once chickens are exposed to the pathogen, the entire flock can become colonized rapidly with *S. Enteritidis* (Foley et al., 2008; Berrang et al., 2009). This could be attributed to the ability of the pathogen to proliferate in the gastrointestinal tract of chicken (Poppe, 2000) and the multitude of sources in farms contributing to pathogen spread in birds. Recent reports reveal that *S. Enteritidis* is invasive in both young and adult chickens (Shah et al., 2011) with young chickens developing systemic disease resulting in death (Duchet-Suchaux et al., 1995; Velge et al., 2005). The affected chicks may show all or some signs such as anorexia, depression, ruffled feathers, huddling together in groups, reluctance to move, drowsiness, dehydration, white diarrhea, stained and pasted vents and stunted growth (McIlroy et al., 1989). However, adult chickens, once colonized with the pathogen may remain as asymptomatic carriers, shedding the pathogen to the environment continuously or intermittently (Velge et al., 2005; Golden et al., 2008). Adult chickens infected with high doses of *S. Enteritidis* can subsequently develop clinical salmonellosis with high mortality, whereas infection with low doses will result in clinically healthy carrier birds (Gast and Benson, 1995; Desmidt et al., 1997; Van Immerseel, 2004a, b).

### **Pre-harvest *Salmonella* control strategy in chickens**

Cecal carriage of *Salmonella* results in horizontal transmission of the pathogen, contamination of eggshells with feces and carcass contamination during slaughter. In slaughterhouses, many opportunities exist for the transfer of pathogens from the outside surface of chickens to the meat during processing of the carcasses. In addition, it is not easy to decrease populations of pathogens on carcasses to less than that existing on the external surfaces of live birds when they arrived at the processing plants (Bailey et al., 1991). Also, mechanical evisceration can cause gastrointestinal spillage of the pathogen, further contaminating the edible carcasses (reviewed by Byrd, 2005). Therefore, substantial reductions in *Salmonella* counts on chicken carcasses can be achieved by delivering birds to the processing plants that are minimally contaminated, or ideally free of these pathogens (Bailey, 1993). Reducing the populations of *Salmonella* in the chicken intestinal tract could reduce contamination of poultry meat and eggs. A variety of approaches for reducing the colonization of *Salmonella*, especially *S. Enteritidis* in chickens has been explored, but with varying degrees of success. These include feeding birds with competitive exclusion bacteria, bacteriophages, organic acids, probiotics, prebiotics, synbiotics and antibiotics (Fernandez et al., 2000; Spring et al., 2000; Byrd et al., 2001; Stern et al., 2001; Fernandez et al., 2002; Chadfield and Hinton, 2003; Heres et al., 2004; Fiorentin et al., 2005; Higgins et al., 2007). Additionally, a number of vaccination approaches to control *S. Enteritidis* in chickens have been undertaken (Barrow, 1997; Dueger et al., 2001; Khan et al.,



2003; Inoue et al., 2008), but there is no fully effective commercial vaccine available currently to prevent colonization of chickens with *Salmonella*. Use of antibiotics, another promising strategy, has been limited due to the issue of emergence of multi-drug resistant strains of *Salmonella*, creating a major public health problem (Shea, 2003; Bywater, 2005). *S. Enteritidis* strains that are resistant to a variety of antibiotics have been reported by several investigators (Chadfield and Hinton, 2003; Daly et al., 2005; Dias de Oliveira et al., 2005; Erdem et al., 2005; Kilmartin et al., 2005). Although some of these pre-harvest approaches have been adopted by the industry, recent outbreaks involving multiple *Salmonella* serotypes via poultry products, especially meat, have triggered research to identify other viable alternatives for *Salmonella* control. Recently there is an emerging interest in using natural and environmentally-friendly approaches to control *Salmonella* colonization in chickens. A few of the potential antimicrobial alternatives are highlighted in this document. Our research on the control of *S. Enteritidis* has been concentrated on three natural compounds, namely caprylic acid (medium chain fatty acid), *trans*-cinnamaldehyde and eugenol (phytphenolics).

### Caprylic acid

Caprylic acid (octanoic acid) is a generally regarded as safe (GRAS; CFR 184.1025) status, natural, eight-carbon medium chain fatty acid present in breast milk, bovine milk (Jensen, 2002) and coconut oil (Jensen et al., 1990, Sprong et al., 2001). In our *in vitro* investigations using sterile chicken cecal contents as a nutrient medium, we observed that caprylic acid rapidly reduced significant populations of *S. Enteritidis* ( $> 5.0 \log \text{CFU}$ ) within 24 hours of incubation. However, caprylic acid was minimally inhibitory towards endogenous cecal population of anaerobic bacteria (Vasudevan et al., 2005). Data from our challenge studies in broilers indicated that caprylic acid was effective against colonization of another poultry borne pathogen, *Campylobacter jejuni* in broiler chickens when supplemented through feed. Solis de los Santos et al. (2008) reported that caprylic acid supplemented at 0.7 and 1.4% to 15 day-old chicks 3 days prior to necropsy resulted in reduction of cecal *C. jejuni* counts by 3 to 5  $\log_{10} \text{CFU/g}$ . Moreover, 0.7% caprylic acid reduced (2 to 3  $\log_{10} \text{CFU/g}$ ) *C. jejuni* colonization consistently in market-age (42-day) broiler chickens when supplemented during 3 or 7 days prior to slaughter (Solis de los Santos et al., 2009).

Similar to our investigations with *C. jejuni* in broiler chickens, we determined the potential of caprylic acid against *S. Enteritidis* (Kollanoor Johny et al., 2009). Chicks challenged with *S. Enteritidis* were given supplemental CA at 0.7 or 1% through feed for 18 days from day 0. Both concentrations of caprylic acid consistently decreased *S. Enteritidis* populations recovered from the cecum of treated birds by 2.5  $\log_{10} \text{CFU/g}$  compared to the controls. Feed intake and body weight did not differ between the treated and control bird groups. Histological examination revealed no pathological changes in the cecum and liver of caprylic acid supplemented birds. The results suggested that prophylactic caprylic acid supplementation through feed could potentially reduce *S. Enteritidis* colonization in chickens (Kollanoor Johny et al., 2009). In the next two trials we determined the therapeutic potential of caprylic acid on cecal *S. Enteritidis* in broilers, when supplemented through feed for the last 5 days before slaughter. Caprylic acid at 0.7 or 1% significantly decreased *S. Enteritidis* populations in the cecum by  $\sim 3 \log_{10} \text{CFU/g}$ , compared to the control birds (Kollanoor Johny et al., 2012c). Further, our cell culture studies revealed that caprylic acid could reduce *S. Enteritidis* invasion of an avian intestinal epithelial cell line and down-regulated the expression of invasion genes, *hilA* and *hilD* (Kollanoor Johny et al., 2012c). These results suggest that supplementation of caprylic acid potentially reduces the pathogen's ability to invade intestinal epithelial cells by down-regulating key invasion genes, *hilA* and *hilD*.



## Trans-cinnamaldehyde

*Trans*-cinnamaldehyde is the major GRAS-status component in the bark extract of cinnamon (21CFR 182.60). It possesses antimicrobial activity towards a wide range of foodborne pathogens, including Gram-positive and Gram-negative bacteria (Bowles and Miller, 1993; Bowles et al., 1995; Friedman et al., 2002). The antibacterial activity of *trans*-cinnamaldehyde against *Clostridium botulinum* (Bowles and Miller, 1993), *Staphylococcus aureus* (Bowles et al., 1995) and *Escherichia coli* O157:H7 has been previously reported. The US Flavoring Extract Manufacturers' Association reported that *trans*-cinnamaldehyde has a wide margin of safety between conservative estimates of intake and no observed adverse effect levels, from sub chronic and chronic studies (Adams et al., 2004). The report also indicated no genotoxic and mutagenic effects due to *trans*-cinnamaldehyde.

We conducted an *in vitro* investigation determining the anti-*Salmonella* potential of a few food grade phytophenolics, including *trans*-cinnamaldehyde (Kollanoor Johny et al., 2010) in a previously described model system (Vasudevan et al., 2005). We observed that *trans*-cinnamaldehyde was highly bactericidal, with the lowest concentration of the compound (0.13%) reducing *S. Enteritidis* populations by  $\sim 6.0 \log_{10}$  CFU/ml after 8 hours and  $> 8.0 \log_{10}$  CFU/ml after 24 hours of incubation. *Trans*-cinnamaldehyde at 0.25% eliminated detectable *S. Enteritidis* counts by 8 hours of incubation (Kollanoor Johny et al., 2010). Follow up in-bird challenge studies were designed to assess the efficacy of supplemental *trans*-cinnamaldehyde in reducing *S. Enteritidis* colonization in broiler chickens. Data from our prophylactic supplementation experiments revealed that *trans*-cinnamaldehyde at 0.5 or 0.75% supplemented for 20 days through feed resulted in 3 to 4  $\log_{10}$  CFU/g reduction of *S. Enteritidis* in the cecum, compared to the control birds (Kollanoor Johny et al., 2012a). *Trans*-cinnamaldehyde did not affect the pH or endogenous cecal microflora counts. Feed intake and body weight were not significantly different for *trans*-cinnamaldehyde supplemented groups. In line with these results, our studies on therapeutic efficacy of *trans*-cinnamaldehyde also revealed that the compound at 0.75% reduced *S. Enteritidis* counts in the cecum of market-age chickens when supplemented through feed for 5 days prior to slaughter (Kollanoor Johny et al., 2012b). Results from our mechanistic studies revealed that *trans*-cinnamaldehyde reduced the motility and invasive abilities of *S. Enteritidis* in cell culture, and down-regulated the expression of invasion genes, *hilA*, *hilD*, and *invF*, and motility genes, *flhC* and *motA* (Kollanoor Johny et al., 2012b).

## Eugenol

Eugenol is a natural GRAS-status (21CFR 582.60) phytophenolic compound present in the clove essential oil (*Eugenia caryophyllis*) (Ali et al., 2005). The antibacterial activity of clove oil and eugenol has been documented by many researchers (Stecchini et al., 1993; Menon and Garg, 2001; Suhr and Nielsen, 2003; Ali et al., 2005). Based on the data from our *in vitro* investigation determining the potential of eugenol on *S. Enteritidis* in autoclaved chicken cecal contents (Kollanoor Johny et al., 2010), we carried out five independent experiments to determine prophylactic and therapeutic efficacies of eugenol in broiler chickens. In the prophylactic study, we observed that 0.75 and 1% eugenol supplemented in feed for 20 days reduced *S. Enteritidis* population in the cecum of broilers significantly (Kollanoor Johny et al., 2012a). Similarly, 1% eugenol supplementation for 5 days prior to slaughter in market age chickens also resulted in similar reduction of *S. Enteritidis* populations (Kollanoor Johny et al., 2012b). Although no toxic effects were observed during short-term therapeutic supplementation in adult birds, the 20-day prophylactic dietary supplementation resulted in reduced body weight. However, no significant difference in the cecal bacterial populations among different treatment groups across the experiments was observed (Kollanoor Johny et al., 2012a, b). Similar to our



observations with *trans*-cinnamaldehyde, our mechanistic studies revealed that eugenol reduced motility and invasion of *S. Enteritidis* in the avian intestinal cell culture, and down-expressed invasion and motility genes (Kollanoor Johny et al., 2012a).

### **Mechanistic basis for antimicrobial activity of natural compounds**

Several mechanisms could be attributed to the antibacterial action of medium chain fatty acids, including caprylic acid. Direct incorporation of caprylic acid, a hydrophobic compound, into the bacterial plasma membrane could compromise membrane permeability (Bergsson et al., 1999). It could diffuse into the bacterial protoplasm and dissociate leading to intracellular acidification affecting enzymes and amino acid transport adversely (Sun et al., 1998; Viegas and Sa-Correia, 1991). Caprylic acid could result in alterations in the cecal microflora populations and change in the physical characteristics of the intestine (Solis de los Santos et al., 2008), although our investigations did not reveal such changes in chickens (Kollanoor Johny et al., 2009; Solis de los Santos et al., 2010). Van Immerseel and coworkers (2004a, b) reported that medium chain fatty acids suppressed the expression of *hilA*, a key gene regulator involved in the invasion of *Salmonella*, thereby resulting in bacterial reduction *in vivo*. Our mechanistic studies also revealed that caprylic acid reduced virulence properties in *S. Enteritidis* by potentially down-regulating critical genes, including *hilA* and *hilD* that control these virulence characteristics.

Similar to medium chain fatty acids, a critical property of phytophenolics is their hydrophobicity that leads to bacterial cell membrane damage and leakage of ions and other cell contents (Knobloch et al., 1986; Sikkema et al., 1994; Smith-Palmer et al., 2004; Cox et al., 2000; Carson et al., 2002; Ultee et al., 2002). Specifically, *trans*-cinnamaldehyde affects glucose uptake and ATP synthesis, two critical growth/survival mechanisms in bacteria (Gill and Holley, 2004). Another mechanism by which phytophenolics kill microorganisms is by inhibiting key enzymes such as amino acid decarboxylases (Wendakoon and Sakaguchi, 1995). Since phytophenolics contain a number of different chemical groups in their structure, their antimicrobial activity is attributed to more than one specific mechanism (Skandamis et al., 2001; Carson et al., 2002; Burt, 2004; Smith-Palmer et al., 2004). In addition to the aforementioned mechanisms of action, *trans*-cinnamaldehyde and eugenol target virulence in *S. Enteritidis*. It was observed that these molecules resulted in reduction of motility and invasion of *S. Enteritidis* in an avian intestinal cell culture model, and down-regulated critical virulence genes (Kollanoor Johny et al., 2012a, b). Moreover, our DNA microarray results indicated that several genes including those involved in the regulation of *Salmonella* Pathogenicity Island 1, type 3 secretion system, outer membrane proteins, metabolic pathways, and electron acceptors under anaerobiosis were down-regulated in *S. Enteritidis* by these molecules (Kollanoor Johny et al., unpublished data).

### **Conclusions and future directions**

Our applied and mechanistic studies indicate that caprylic acid, *trans*-cinnamaldehyde and eugenol could be potential candidates as in-feed antimicrobials against *S. Enteritidis* colonization in poultry. These molecules significantly reduced cecal colonization of the pathogen in young and market age birds. In addition to the direct antimicrobial action, these molecules were able to reduce virulence properties in *S. Enteritidis*, including motility, invasion, systemic survival, expression of outer-membrane proteins, and utilization of alternate energy sources for survival and growth in anaerobic conditions. The inhibition of multiple virulence pathways in *Salmonella* without adversely affecting the birds could be seen as unique about these molecules that may qualify them for inclusion in commercial poultry diets. Long-term stability studies in feed and economic considerations for inclusion in poultry feeds need to be explored further. Currently, investigations determining the potential of these molecules on reducing egg-borne transmission of *S. Enteritidis* in layer chickens are in its final stages.



## Acknowledgements

The authors thankfully acknowledge the USDA for funding the research highlighted in this document through grants, USDA-NIFA-OREI 2011-01955, USDA-AFRI 2009-03576 and USDA-NIFSI 2006-02429.

## References

- Adams, T. B., S. M. Cohen, J. Doull, V. J. Feron, J. I. Goodman, L. J. Marnett, I. C. Munro, P. S. Portoghese, R. L. Smith, W. J. Waddell, and B. M. Wagner. 2004. The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. *Food Chem. Toxicol.* 42:157-185.
- Ali, S. M., A. A. Khan, I. Ahmed, M. Musaddiq, K. S. Ahmed, H. Polasa, V. L. Rao, C. M. Habibullah, L. A. Sechi, and N. Ahmed. 2005. Antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. *Ann. Clin. Microbiol. Antimicrob.* 4:20-26.
- Altekruse, S. F., N. Bauer, A. Chanlongbutra, R. DeSagun, A. Naugle, W. Schlosser, R. Umholtz, and P. White. 2006. *Salmonella* Enteritidis in broiler chickens, United States, 2000–2005. *Emerg. Infect. Dis.* 12:1848-1852.
- Bailey, J. S. 1993. Control of *Salmonella* and *Campylobacter* in poultry production. A summary of Russell research center. *Poultry Sci.* 72:1169-1173.
- Bailey, J. S., L. C. Blankenship, and N. A. Cox. 1991. Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. *Poultry Sci.* 70:2433-2438.
- Barrow, P. A. 1997. Novel approaches to control of bacterial infections in animals. *Acta. Vet. Hung.* 45:317-329.
- Bergsson, G., O. Steingrímsson, and H. Thormar. 1999. *In vitro* susceptibilities of *Neisseria gonorrhoeae* to fatty acids and monoglycerides. *Antimicrob. Agents Chemother.* 43:2790-2792.
- Berrang, M. E., J. S. Bailey, S. F. Altekruse, W. K. Shaw, B. L. Patel, R. J. Meinersmann, and P. J. Fedorka-Cray. 2009. Prevalence, serotype, and antimicrobial resistance of *Salmonella* on broiler carcasses postpick and postchill in 20 U.S. processing plants. *J. Food Prot.* 72:1610-1610.
- Bowles, B. L., and A. J. Miller. 1993. Antibotulinal properties of selected aromatic and aliphatic aldehydes. *J. Food Prot.* 56:788–794.
- Bowles, B. L., S. K. Sackitey, and A. C. Williams. 1995. Inhibitory effects of flavor compounds on *Staphylococcus aureus* WRC B124. *J. Food Saf.* 15:337–347.
- Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in food – A review. *Intl. J. Food Microbiol.* 94:223–253.
- Byrd, J. A. 2005. Improving slaughter and processing technologies. In: Food safety control in the poultry industry. G. Mead (ed.) Woodhead Publishing, Cambridge, UK, Pp 310-332.
- Byrd, J. A., B. M. Hargis, D. J. Caldwell, R. H. Bailey, K. L. Herron, J. L. McReynolds, R. L. Brewer, R. C. Anderson, K. M. Bischoff, T. R. Callaway, and L. F. Kubena. 2001. Effect of lactic acid administration in the drinking water during pre-slaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. *Poultry Sci.* 80:278-283.



- Bywater, R. J. 2005. Identification and surveillance of antimicrobial resistance dissemination in animal production. *Poultry Sci.* 84:644-648.
- Carson, C. F., B. J. Mee, and T. V. Riley. 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob. Agents Chemother.* 46:1914-1920.
- CDC, 2011. Surveillance for foodborne disease outbreaks- United States, 2008. *Morb. Mort. Weekly Rep.* 60:1197-1202.
- CDC. 2014. Outbreak of *Salmonella* Heidelberg Infections Linked to Tyson Brand Mechanically Separated Chicken at a Correctional Facility. Access online at <http://www.cdc.gov/salmonella/heidelberg-01-14/>
- Chadfield, M. S. and M. H. Hinton. 2003. Evaluation of treatment and prophylaxis with nitrofurans and comparison with alternative antimicrobial agents in experimental *Salmonella enterica* serovar Enteritidis infection in chicks. *Vet. Res. Commun.* 27:257-273.
- Clavijo, R. I., C. Loui, G. L. Andersen, L. W. Riley, and S. Lu S. 2006. Identification of genes associated with survival of *Salmonella enterica* serovar Enteritidis in chicken egg albumen. *Appl. Environ. Microbiol.* 72:1055-1064.
- Cox, S. D., C. M. Mann, J. L. Markham, H. C. Bell, J. E. Gustafson, J. R. Warmington, and S. G. Wyllie. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.* 88:170-175.
- Daly, M., L. Villa, C. Pezzella, S. Fanning, and A. Carattoli. 2005. Comparison of multidrug resistance gene regions between two geographically unrelated *Salmonella* serotypes. *J. Antimicrob. Chemother.* 55:558-561.
- D'Aoust, J. Y. 1991. Pathogenicity of foodborne *Salmonella*. *Intl. J. Food Microbiol.* 12:17-40.
- Desmidt, M., R. Ducatelle, and F. Haesebrouck. 1997. Pathogenesis of *Salmonella* Enteritidis phage type four after experimental infection of young chickens. *Vet. Microbiol.* 56: 99-109.
- Dhawi, A. A., A. Elazomi, M. A. Jones, M. A. Lovell, H. Li, R. D. Emes, and P. A. Barrow. 2011. Adaptation to the chicken intestine in *Salmonella* Enteritidis PT4 studied by transcriptional analysis. *Vet. Microbiol.* 153: 198-204.
- Dias de Oliveira, S., F. Siqueira Flores, L. R. dos Santos, and A. Brandelli. 2005. Antimicrobial resistance in *Salmonella* Enteritidis strains isolated from broiler carcasses, food, human and poultry-related samples. *Intl. J. Food Microbiol.* 97:297-305.
- Duchet-Suchaux M., P. Lechopier, J. Marly, P. Bernardet, R. Delaunay, and P. Pardon. 1995. Quantification of experimental *Salmonella* Enteritidis carrier state in B13 leghorn chicks. *Avian dis.* 39:796-803.
- Dueger, E. L., J. K. House, D. M. Heithoff, and M. J. Mahan. 2001. *Salmonella* DNA adenine methylase mutants elicit protective immunity responses to homologous serovars in chickens. *Infect. Immun.* 69:7950-7954.
- Erdem, B., S. Ercis, G. Hascelik, D. Gur, S. Gedikoglu, A. D. Aysev, B. Sumerkan, M. Tatman-Otkun, and I. Tuncer. 2005. Antimicrobial resistance patterns and serotype distribution among *Salmonella enterica* strains in Turkey, 2000-2002. *Eur. J. Clin. Microbiol. Infect. Dis.* 24:220-225.
- Fernandez, F., M. Hinton, and B. van Gils. 2000. Evaluation of the effect of mannan-oligosaccharides on the competitive exclusion of *Salmonella* Enteritidis colonization in broiler chicks. *Avian Pathol.* 29:575-581.



- Fernandez, F., M. Hinton, and B. van Gils. 2002. Dietary mannan-oligosaccharides and their effect on chicken cecal microflora in relation to *Salmonella* Enteritidis colonization. *Avian Pathol.* 31:49-58.
- Fiorentin, L., N. D. Vieira, and W. Barioni, Jr. 2005. Oral treatment with bacteriophages reduces the concentration of *Salmonella* Enteritidis PT4 in caecal contents of broilers. *Avian Pathol.* 34:258-263.
- Foley, S. L., A. M. Lynne, and R. Nayak. 2008. *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *J. Animal Sci.* 86:E149-E162.
- Food Safety News, 2013. Access online at <http://www.foodsafetynews.com/2013/10/mexico-bans-foster-farms-chicken-from-plants-linked-to-outbreak/#.Uu8dRPIdV8E>
- Friedman, M., P. R. Henika, and R. E. Mandrell. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* 65:1545-1560.
- Gast, R. K. and S. T. Benson. 1995. The comparative virulence for chicks of *Salmonella* Enteritidis phage type 4 isolates and isolates of phage types commonly found in poultry in the United States. *Avian Dis.* 39:567-574.
- Gennis, R. B. and V. Stewart, 1996. *Escherichia coli* and *Salmonella*—Cellular and Molecular Biology. ASM Press, Washington, DC.
- Gill, A. O. and R. A. Holley. 2004. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *Intl. J. Food Microbiol.* 108:1-9.
- Golden, N. J., H. H. Marks, M. E. Coleman, C. M. Schroeder, N. E. Bauer Jr., and W. D. Schlosser 2008. Review of induced molting by feed removal and contamination of eggs with *Salmonella enterica* serovar Enteritidis. *Vet. Microbiol.* 131:215-228.
- Heres, L., B. Engel, H. A. P. Urlings, J. A. Wagner, and F. van Knapen. 2004. Effect of acidified feed on susceptibility of broiler chickens to intestinal infection by *Campylobacter* and *Salmonella*. *Vet. Microbiol.* 99:259-267.
- Higgins, S. E., G. F. Erf, J. P. Higgins, S. N. Henderson, A. D. Wolfenden, G. Gaona-Ramirez, and B. M. Hargis. 2007. Effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella* Enteritidis by abdominal exudate cells. *Poult Sci.* 86:2315-2321.
- Humphrey, T., and Jorgensen, F. 2006. Pathogens on meat and infection in animals: establishing a relationship using *Campylobacter* and *Salmonella* as examples. *Meat Sci.* 74: 89-97.
- Inoue, A. Y., A. Berchieri, Jr., A. Bernardino, J. B. Paiva, and E. V. Sterzo. 2008. Passive immunity of progeny from broiler breeders vaccinated with oil-emulsion bacterin against *Salmonella* Enteritidis. *Avian Dis.* 52: 567-571.
- Jackson, B. R., P. M. Griffin, D. Cole, K. A. Walsh, and S. J. Chai. 2013. Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. *Emerging Infect. Dis.* 19:1239-1244.
- Jain, S. and J. Chen. 2007. Attachment and biofilm formation by various serotypes of *Salmonella* as influenced by cellulose production and thin aggregative fimbriae biosynthesis. *J. Food Prot.* 70:2473-2479.



- Jensen, R. G., A. M. Ferris, C. J. Lammi-Keefe, and R.A. Henderson. 1990. Lipids of bovine and human milks: a comparison. *J. Dairy Sci.* 73:223-240.
- Jensen, R.G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. *J. Dairy Sci.* 85:295-350.
- Khan, M. I., A. A. Fadl, and K. S. Venkitanarayanan. 2003. Reducing colonization of *Salmonella* Enteritidis in chicken by targeting outer membrane proteins. *J. Appl. Microbiol.* 95:142-145.
- Kilmartin, D., D. Morris, C. O'Harre, G. Corbett-Feeney, and M. Cormican. 2005. Clonal expansion may account for high levels of quinolone resistance in *Salmonella enterica* Seroovar Enteritidis. *Appl. Environ. Microbiol.* 71:2587-2591.
- Knobloch, K., H. Weigand, N. Weis, H. M. Schwarm, and H. Vogenschow. 1986. Action of terpenoids on energy metabolism. In: Brunke, E.J. (Ed.), *Progress in Essential Oil Research: 16th International Symposium on Essential Oils*. Berlin. Pp 429-445.
- Kollanoor-Johny, A., S. A. Baskaran, A. S. Charles, M. A. R. Amalaradjou, M. J. Darre, M. I. Khan, T. A. Hoagland, D. T. Schreiber, A. M. Donoghue, D. J. Donoghue, and Venkitanarayanan, K. 2009. Prophylactic supplementation of caprylic acid in feed reduces *Salmonella* Enteritidis population in commercial broiler chicks. *J. Food Prot.* 72:722-727.
- Kollanoor-Johny, A., T. Mattson, S. A. Baskaran, M. A. Amalaradjou, S. Babapoor, B. March, S. Valipe, M. Darre, T. Hoagland, D. Schreiber, M. I. Khan, A. Donoghue, D. Donoghue, and K. Venkitanarayanan. 2012a. Reduction of *Salmonella enterica* Seroovar Enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds *trans*-cinnamaldehyde and eugenol. *Appl. Environ. Microbiol.* 78: 2981-2987.
- Kollanoor-Johny, A., M. J. Darre, A. M. Donoghue, D. J. Donoghue, and K. Venkitanarayanan. 2010. Antibacterial effect of *trans*-cinnamaldehyde and thymol on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken cecal contents *in vitro*. *J. Appl. Poultry Res.* 19: 237-244.
- Kollanoor-Johny, A., T. Mattson, S. A. Baskaran, M. A. R. Amalaradjou, M. J. Darre, D. T. Schreiber, M. I. Khan, A. M. Donoghue, D. J. Donoghue, and K. Venkitanarayanan. 2012c. Caprylic acid reduces *Salmonella* Enteritidis populations in various segments of digestive tract and internal organs of three- and six-week-old broiler chickens, therapeutically. *Poultry Sci.* 91:1686-1694.
- Kollanoor-Johny, A., A. Upadhyay, S. A. Baskaran, I. Upadhyaya, S. Mooyottu, N. Mishra, M. J. Darre, M. I. Khan, A. M. Donoghue, D. J. Donoghue, and K. Venkitanarayanan. 2012b. Effect of therapeutic supplementation of the plant compounds *trans*-cinnamaldehyde and eugenol on *Salmonella enterica* serovar Enteritidis colonization in market-age broiler chickens. *J. Appl. Poultry Res.* 21: 816-822.
- Marcus, R., J. K. Varma, C. Medus, E. J. Boothe, B. J. Anderson, T. Crume, K. E. Fullerton, M. R. Moore, P. L. White, E. Lyszkowicz, A. C. Voetsch, and F. J. Angula. 2007. Re-assessment of risk factors for sporadic *Salmonella* serotype Enteritidis infections: a case-control study in five FoodNet sites, 2002-2003. *Epidemiol. Infect.* 135:84-92.
- McIlroy, S. G., R. M. McCracken, S. D. Neill, and J. J. O'Brien. 1989. Control, Prevention, and eradication of *Salmonella* Enteritidis infection in broiler and broiler breeder flocks. *Vet. Rec.* 125: 545-548.



- Menon, K. V. and S.R. Garg. 2001. Inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese. *Food Microbiol.* 18:647-650.
- Poppe, C. 2000. *Salmonella* infections in the domestic fowl. In: *Salmonella* in domestic animals. Wray, C., and A. Wray (Eds.). CABI Publishing: Wallingford, UK. Pp: 194.
- Porwollik, S., C. A. Santiviago, P. Cheng, L. Florea, and M. McClelland. 2005. Differences in gene content between *Salmonella enterica* serovar Enteritidis isolates and comparison to closely related serovars Gallinarum and Dublin. *J. Bacteriol.* 187:6545-6555.
- Poulsen, L. K., T. R. Licht, C. Rang, K. A. Krogfelt, and S. Molin. 1995. Physiological state of *Escherichia coli* BJ4 growing in the large intestines of streptomycin-treated mice. *J. Bacteriol.* 177:5840-5845.
- Pullinger, G. D., F. Dziva, B. Charleston, T. S. Wallis, and M. P. Stevens. 2008. Identification of *Salmonella enterica* serovar Dublin-specific sequences by subtractive hybridization and analysis of their role in intestinal colonization and systemic translocation in cattle. *Infect. Immun.* 76:5310-5321.
- Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States major pathogens. *Emerging Infect. Dis.* 17: 7-15.
- Scharff, R. L. 2012. Economic burden from health losses due to foodborne illness in the United States. *J. Food Prot.* 75: 123–131.
- Shah, D. H., X. Zhou, T. Addwebi, M. A. Davis, L. Orfe, D. R. Call, J. Guard, and T. E. Besser. 2011. Cell invasion of poultry-associated *Salmonella enterica* serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. *Microbiol.* 157:1428-1428.
- Shea, K. M. 2003. Antibiotic resistance: what is the impact of agricultural uses of antibiotics on children's health? *Pediatr.* 112:253-258.
- Sikkema, J., J. A. M. De Bont, and B. Poolman. 1994. Interactions of cyclic hydrocarbons with biological membranes. *J. Biol. Chem.* 269:8022–8028.
- Skandamis, P. N. and G. J. E. Nychas. 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *J. Appl. Microbiol.* 91:1011-1022.
- Slauch, J., R. Taylor, and S. Maloy. 1997. Survival in a cruel world: how *Vibrio cholerae* and *Salmonella* respond to an unwilling host. *Gene Dev.* 11:1761-1774.
- Smith-Palmer, A., J. Stewart, and L. Fyfe. 2004. Influence of subinhibitory concentrations of plant essential oils on the production of enterotoxins A and B and alpha toxin by *Staphylococcus aureus*. *J. Med. Microbiol.* 53:1023-1027.
- Solis de los Santos, F., A. M. Donoghue, K. Venkitanarayanan, I. Reyes-Herrera, J. H. Metcalf, M. L. Dirain, V. F. Aguiar, P. J. Blore, and D. J. Donoghue. 2008. Therapeutic supplementation of caprylic acid in feed reduces *Campylobacter jejuni* colonization in broiler chicks. *Appl. Environ. Microbiol.* 74:4564–4566.
- Solis de los Santos, F., A. M. Donoghue, K. Venkitanarayanan, J. H. Metcalf, I. Reyes-Herrera, M. L. Dirain, V. F. Aguiar, P. J. Blore, and D. J. Donoghue. 2009. The natural feed additive caprylic acid decreases *Campylobacter jejuni* colonization in market-aged broiler chickens. *Poultry Sci.* 88:61-64.



- Solís de los Santos, F., M. Hume, K. Venkitanarayanan, A. M. Donoghue, I. Hanning, M. F. Slavik, V. F. Aguiar, J. H. Metcalf, I. Reyes-Herrera, P. J. Blore, and D. J. Donoghue. 2010. Caprylic Acid reduces enteric campylobacter colonization in market-aged broiler chickens but does not appear to alter cecal microbial populations. *J. Food Prot.* 73: 251-257.
- Spring, P., C. Wenk, K. A. Dawson, and K. A. Newman. 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the caeca of *Salmonella*-challenged broiler chicks. *Poultry Sci.* 79:205-211.
- Sprong, R. C., M. F. Hulstein, and R. Van Der Meer. 2001. Bactericidal activities of milk lipids. *Antimicrob. Agents Chemother.* 45:1298-1301.
- Stecchini, M. L., I. Sarais, and P. Giavedoni. 1993. Effect of essential oils on *Aeromonas hydrophila* in a culture medium and in cooked pork. *J. Food Prot.* 56:406-409.
- Stern, N. J., N. A. Cox, J. S. Bailey, M. E. Berrang, and M. T. Musgrove. 2001. Comparison of mucosal competitive exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter* spp. colonization in broiler chickens. *Poultry Sci.* 80:156-160.
- Suhr, K. and P. V. Nielsen. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *J. Appl. Microbiol.* 94:665-674.
- Sun, C. Q., C. J. O'Connor, S. J. Turner, G. D. Lewis, R. A. Stanley, and A. M. Robertson. 1998. The effect of pH on the inhibition of bacterial growth by physiological concentrations of butyric acid: implications for neonates fed on suckled milk. *Chem. Biol. Interact.* 113:117-131.
- Swaminathan, B., T. J. Barrett, S. B. Hunter, R. V. Tauxe, and C.D.C. Pulse Net Task Force. 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerg. Infect. Dis.* 7:382-389.
- Travers, K. and M. Barza. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. *Clin. Infect. Dis.* 34:S131-S134.
- Ultee, A., M. H. J. Bennink, and R. Moezelaar. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* 68:1561-1568.
- US Poultry and Egg Association. 2014. Economic Data. Accessed online at [http://www.uspoultry.org/economic\\_data/](http://www.uspoultry.org/economic_data/)
- USDA. 2009. Economic Research Service. ERS/USDA Data Foodborne Illness Cost Calculator.
- Van Immerseel, F., J. De Buck, F. Boyen, L. Bohez, F. Pasmans, J. Volf, M. Sevcik, I. Rychlik, F. Haesebrouck, and R. Ducatelle, R. 2004b. Medium-chain fatty acids decrease colonization and invasion shortly after infection with *Salmonella* Enteritidis in chickens through *hilA* suppression. *Appl. Environ. Microbiol.* 70:3582-3587
- Van Immerseel, F., J. De Buck, F. Pasmans, L. Bohez, F. Boyen, F. Haesebrouck, and R. Ducatelle. 2004a. intermittent long-term shedding and induction of carrier birds after infection of chickens early posthatch with a low or high dose of *Salmonella* Enteritidis. *Poultry Sci.* 83:1911-1916.
- Vasudevan, P., M. Patrick, M. Nair, T. Annamalai, M. Darre, M. Khan, and K. Venkitanarayanan. 2005. *In vitro* inactivation of *Salmonella* Enteritidis in chicken cecal contents by caprylic acid. *J. Appl. Poultry Res.* 14:122-125.

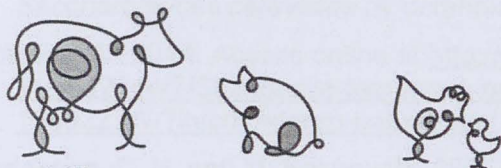


- Velge, P., A. Cloeckaert, and P. Barrow. 2005. Emergence of *Salmonella* epidemics: The problems related to *Salmonella enterica* serotyp Enteritidis and multiple antibiotic resistance in other major serotypes. *Vet. Res.* 36:267-288.
- Viegas, C. A., and I. Sa-Correia. 1991. Activation of plasma membrane ATPase of *Saccharomyces cerevisiae* by octanoic acid. *J. Gen. Microbiol.* 137: 45-651.
- Vorotnikov, V. 2014. Access online at <http://www.worldpoultry.net/Broilers/Markets--Trade/2014/7/Salmonella-fears-may-prompt-Russian-ban-of-US-poultry-1569223W/?intcmp=terug-naar-artikel>
- Wendakoon, C. N. and M. Sakaguchi. 1995. Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. *J. Food Prot.* 56:410-413.
- White, P. L., A. L. Naugle, C. R. Jackson, P. J. Fedorka-Cray, B. E. Rose, K. M. Pritchard, P. Levine, P. K. Saini, C. M. Schroeder, M. S. Dreyfuss, R. Tan, K. G. Holt, J. Harman, and S. Buchanan. 2007. *Salmonella* Enteritidis in meat, poultry and pasteurized egg products regulated by the US Food Safety and Inspection Service, 1998 through 2003. *J. Food Prot.* 70: 582–591.
- Yim, L., L. Betancor, A. Martinez, G. Giossa, C. Bryant, D. Maskell, and J. A. Chabalgoity. 2010. Differential phenotypic diversity among epidemic-spanning *Salmonella enterica* serovar Enteritidis isolates from humans or animals. *Appl. Environ. Microbiol.* 76:6812-6820.







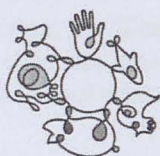


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